MEMORANDUM

Subject: Trifluralin Tier II Seed Germination Testing, DP #D189443.

To: Walter Waldrop, PM 71
    Special Review and Reregistration Division

From: Anthony Maciorowski, Chief
      Ecological Effects Branch
      Environmental Fate and Effects Division, H7507C

EEB has completed a review of the Tier II Seed Germination Testing for Trifluralin; the following is a summary of that review:


This study is scientifically sound and fulfills the guideline requirements for a Tier 2 germination study; Tier I testing had previously determined that only cabbage and onion needed to be tested at the Tier 2 level. Onion radicle length was the most sensitive parameter with NOEC, LOEC, EC$_{25}$, and EC$_{50}$ values of 0.13, 0.25, 0.33, and 4.3 lb ai/A, respectively. The EC$_{25}$ and EC$_{50}$ for cabbage germination and the EC$_{50}$ for onion germination should be considered as >4.0 lb ai/A Table 4 (attached). The NOEC and reasonable EC$_{25}$ estimate for onion germination are 2 and 4 lb ai/A, respectively.

Questions regarding this review, please contact Dana Lateulere at 308-2856.
DATA EVALUATION RECORD

1. CHEMICAL: Trifluralin.
   Shaughnessy No. 036101.

2. TEST MATERIAL: Trifluralin technical; Lot No. 428APO; 95%
   active ingredient; an orange solid.

3. STUDY TYPE: 123-1. Non-Target Plants: Seed Germination
   Phytotoxicity Test - Tier 2. Species Tested: Cabbage and
   Onion.

4. CITATION: Schwab, D. 1993. Evaluating the Effects of
   Trifluralin on the Germination of Non-Target Terrestrial
   Plants. Laboratory Project ID No. 40619. Conducted by
   Analytical Bio-Chemistry Laboratories, Inc., Columbia, MO.
   Submitted by DowElanco, Indianapolis, IN. EPA MRID No.
   426956-01.

5. REVIEWED BY:
   Dana Lateulere, Biologist
   [Edited KBN submittal:
   Mark Mossler, 4/26/93.]

6. APPROVED BY:
   Ann Stavola, Section Head

7. CONCLUSIONS: This study is scientifically sound and
   fulfills the guideline requirements for a Tier 2 germination
   study; Tier I testing had previously determined that only
   cabbage and onion needed to be tested at the Tier 2 level.
   Onion radicle length was the most sensitive parameter with
   NOEC, LOEC, EC₂₅, and EC₉₀ values of 0.13, 0.25, 0.33, and 4.3
   lb ai/A, respectively. The EC₂₅ and EC₉₀ for cabbage
   germination and the EC₉₀ for onion germination should be
   considered as >4.0 lb ai/A Table 4 (attached). The NOEC and
   reasonable EC₂₅ estimate for onion germination are 2 and 4 lb
   ai/A, respectively.

8. RECOMMENDATIONS: N/A.

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.
DATA EVALUATION RECORD

1. **CHEMICAL:** Trifluralin.
   Shaughnessey No. 036101.

2. **TEST MATERIAL:** Trifluralin technical; Lot No. 428APO; 95% active ingredient; an orange solid.

3. **STUDY TYPE:** 123-1. Non-Target Plants: Seed Germination Phytotoxicity Test - Tier 2. Species Tested: Cabbage and Onion.


5. **REVIEWED BY:**
   Mark A. Mossier, M.S.
   Associate Scientist
   KBN Engineering and
   Applied Sciences, Inc.
   
   **Signature:**
   **Date:** 4/26/93

6. **APPROVED BY:**
   Pim Kosalwat, Ph.D.
   Senior Scientist
   KBN Engineering and
   Applied Sciences, Inc.
   
   **Signature:**
   **Date:** 10/5/93

7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements. The required number of test species (i.e., 10 species) was not treated with the test material. Onion radicle length was the most sensitive parameter with NOEC, LOEC, EC_{25}, and EC_{50} values of 0.13, 0.25, 0.33, and 4.3 lb ai/A, respectively.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**
11. MATERIALS AND METHODS:

A. **Test Plants:** Dicotyledon plants were represented by one species from one family (i.e., cabbage) and monocotyledon plants were represented by one species from one family (i.e., onion). Cultivars, seed sources, lot numbers, and germination ratings were provided in the report.

B. **Test System:** Two circles of filter paper were placed in the bottom of a 9-cm disposable petri plate. Seven milliliters of the test solution were added to each plate. Ten seeds of each crop were added to each petri plate after the test solution was absorbed into the paper. The plates were wrapped with moistened filter paper and randomly placed in an environmental chamber set to maintain the temperature at 25 ±2°C. The relative humidity in the chamber was greater than 90%. Seeds were incubated for 5 days.

C. **Dosage:** The test material was applied at the rates of 0.125, 0.25, 0.5, 1.0, 2.0, and 4.0 lb active ingredient (ai)/acre (A). Onion was also treated at the rate of 0.0313 and 0.0625 lb ai/A.

The test solutions were prepared in a 2.8% acetone/deionized water mixture. The maximum application rate of trifluralin was reported to be 8 lb ai/A.

D. **Design:** Each crop/treatment combination was replicated four times (i.e., 10 seeds/plate, 4 plates/treatment level). After incubation, germinated seeds were removed from the petri plates and radicle length determined. A seed with a radicle length of 5 mm was considered germinated. Observations of seedling health were also made.

E. **Statistics:** All data were entered into a computer spreadsheet. The spreadsheet calculated replicate means, treatment means, and standard deviations. Treatment means were used to calculate the percent effect resulting from the treatment. The percent effect was calculated using the following equation:

\[ \% \text{ effect} = \left( \frac{\text{control mean} - \text{treatment mean}}{\text{control mean}} \right) \times 100 \]

Percent germination and root length data were analyzed using analysis of variance (ANOVA). Treatment means were compared to control means by Dunnett’s comparison
test (95% confidence level). The percentage data were arcsine transformed before the analysis.

If a significant difference was detected by Dunnett's test, and a dose related response of 50% or more was observed, then probit analysis or non-linear regression was used to determine the EC values.

12. **REPORTED RESULTS:** Root lesions and necrosis were observed sporadically on cabbage exposed to trifluralin, but these observations were not considered to be treatment-related. For onion, swelling of the roots and shoots were observed in plants in the three highest concentration solutions. Fungus was observed in all treatments, but was not considered to be treatment-related.

**Percent Germination:** The no-observed-effect level (NOEL) for cabbage and onion were determined to be 6 and >12 µg ai/ml (2 and >4 lb ai/A), respectively. The EC values could not be calculated due the lack of a dose response for both species.

**Radicle Length:** The NOEC, EC$_{25}$, and EC$_{50}$ for radicle length of cabbage were 3.0, 4.8, and 13 µg ai/ml (1, 1.6, and 4.3 lb ai/A), respectively.

The NOEC, EC$_{25}$, and EC$_{50}$ for radicle length of onion were 0.38, 1.0, and 13 µg ai/ml (0.13, 0.33, and 4.3 lb ai/A), respectively.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions other than those previously stated were made by the author.

The Quality Assurance Unit of ABC Laboratories, Inc., indicated that the data collection and report were accurate reflections of the study as it was conducted. A statement of Compliance with Good Laboratory Practice Standards was provided.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedures followed the SEP and Subdivision J guidelines.

Although a negative control was not included in the study design, the percentage germination (88-93%) indicated that the solvent system had little effect on control seed germination.
B. **Statistical Analysis:** Probit analysis was conducted on onion radicle length (the most sensitive species) data to determine the EC values. The ANOVA (coupled with Dunnett's test) was used to verify the NOEL and lowest-observed-effect level (LOEL). Since the author's model predicted a more conservative EC\textsubscript{25}, the results from this model will be used rather than the reviewer's. Results from ANOVA were in agreement with the author's (see attached printouts).

C. **Discussion/Results:** The EC\textsubscript{25} and EC\textsubscript{50} for cabbage germination and the EC\textsubscript{50} for onion germination should be considered as >4.0 lb ai/A Table 4 (attached). The NOEC and reasonable EC\textsubscript{25} estimate for onion germination are 2 and 4 lb ai/A, respectively. Onion radicle length was the most sensitive parameter with NOEC, LOEC, EC\textsubscript{25}, and EC\textsubscript{50} values of 0.13, 0.25, 0.33, and 4.3 lb ai/A, respectively.

D. **Adequacy of the Study:**

1. **Classification:** Core.

2. **Rationale:** n/a.

3. **Repairability:** n/a.
TABLE 4  EC$_{25}$, and EC$_{50}$ and NOEC Values in µg a.i./mL Obtained from the Germination Test with Trifluralin

<table>
<thead>
<tr>
<th>Species</th>
<th>EC$_{25}$ (µg a.i./mL)</th>
<th>EC$_{50}$ (µg a.i./mL)</th>
<th>NOEC (µg a.i./mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cabbage Germination</td>
<td>&gt;1.2 ND</td>
<td>&gt;1.2 ND</td>
<td>1.2 ND</td>
</tr>
<tr>
<td>Onion Germination</td>
<td>~1.2 ND</td>
<td>&gt;1.2 ND</td>
<td>6.0</td>
</tr>
<tr>
<td>Cabbage Root Length</td>
<td>4.8</td>
<td>13</td>
<td>3.0</td>
</tr>
<tr>
<td>Onion Root Length</td>
<td>1.0</td>
<td>13</td>
<td>0.38</td>
</tr>
</tbody>
</table>

ND = Not Determined
onion radicle length
File: oni Transform: NO TRANSFORM

## ANOVA TABLE

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>8</td>
<td>341.222</td>
<td>42.653</td>
<td>9.597</td>
</tr>
<tr>
<td>Within (Error)</td>
<td>27</td>
<td>120.000</td>
<td>4.444</td>
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</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>461.222</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Critical F value = 2.31 (0.05,8,27)
Since F > Critical F REJECT Ho:All groups equal

## DUNNETTS TEST - TABLE 1 OF 2

Ho:Control<Treatment

<table>
<thead>
<tr>
<th>GROUP</th>
<th>IDENTIFICATION</th>
<th>TRANSFORMED MEAN</th>
<th>MEAN CALCULATED IN ORIGINAL UNITS</th>
<th>T STAT</th>
<th>SIG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>control</td>
<td>17.000</td>
<td>17.000</td>
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<td></td>
</tr>
<tr>
<td>2</td>
<td>0.031</td>
<td>15.750</td>
<td>15.750</td>
<td>0.839</td>
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</tr>
<tr>
<td>3</td>
<td>0.063</td>
<td>17.000</td>
<td>17.000</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.125</td>
<td>15.000</td>
<td>15.000</td>
<td>1.342</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.25</td>
<td>11.250</td>
<td>11.250</td>
<td>3.857*</td>
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</tr>
<tr>
<td>6</td>
<td>0.5</td>
<td>11.250</td>
<td>11.250</td>
<td>3.857*</td>
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</tr>
<tr>
<td>7</td>
<td>1</td>
<td>14.500</td>
<td>14.500</td>
<td>1.677</td>
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</tr>
<tr>
<td>8</td>
<td>2</td>
<td>9.000</td>
<td>9.000</td>
<td>5.367*</td>
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</tr>
<tr>
<td>9</td>
<td>4</td>
<td>8.750</td>
<td>8.750</td>
<td>5.534*</td>
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</tr>
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</table>

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=24,8)

## DUNNETTS TEST - TABLE 2 OF 2

Ho:Control<Treatment

<table>
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<tr>
<th>GROUP</th>
<th>IDENTIFICATION</th>
<th>NUM OF REPS</th>
<th>Minimum Sig Diff</th>
<th>% of Difference</th>
<th>DIFFERENCE FROM CONTROL</th>
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<tbody>
<tr>
<td>1</td>
<td>control</td>
<td>4</td>
<td>3.772</td>
<td>22.2</td>
<td>1.250</td>
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<tr>
<td>2</td>
<td>0.031</td>
<td>4</td>
<td>3.772</td>
<td>22.2</td>
<td>0.000</td>
</tr>
<tr>
<td>3</td>
<td>0.063</td>
<td>4</td>
<td>3.772</td>
<td>22.2</td>
<td>0.000</td>
</tr>
<tr>
<td>4</td>
<td>0.125</td>
<td>4</td>
<td>3.772</td>
<td>22.2</td>
<td>2.000</td>
</tr>
<tr>
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<td>4</td>
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<td>4</td>
<td>4</td>
<td>3.772</td>
<td>22.2</td>
<td>8.250</td>
</tr>
</tbody>
</table>
onion radicle length

Estimated EC Values and Confidence Limits

<table>
<thead>
<tr>
<th>Point</th>
<th>Conc.</th>
<th>Lower 95% Confidence Limits</th>
<th>Upper 95% Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC 1.00</td>
<td>0.0041</td>
<td>0.0006</td>
<td>0.0128</td>
</tr>
<tr>
<td>EC 5.00</td>
<td>0.0304</td>
<td>0.0089</td>
<td>0.0648</td>
</tr>
<tr>
<td>EC10.00</td>
<td>0.0886</td>
<td>0.0364</td>
<td>0.1563</td>
</tr>
<tr>
<td>EC15.00</td>
<td>0.1824</td>
<td>0.0927</td>
<td>0.2880</td>
</tr>
<tr>
<td>EC50.00</td>
<td>3.8610</td>
<td>2.4576</td>
<td>7.5184</td>
</tr>
<tr>
<td>EC85.00</td>
<td>81.7305</td>
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<tr>
<td>EC90.00</td>
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<td>EC95.00</td>
<td>490.6064</td>
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<td>EC99.00</td>
<td>3650.3201</td>
<td>638.4584</td>
<td>68653.8910</td>
</tr>
</tbody>
</table>

\[ y = 4.5x + 0.78(x) \]

\[ y = \text{percent inhibition} \]

\[ x = \log (\text{EC}) \]

EC<sub>25</sub> = 0.54 / 16 ai/A